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The Use of Blood and Derived Products as Food Additives

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1. Introduction

Blood is a rich source of iron and proteins of high nutritional and functional quality. Because of the high protein content of blood, generally about 18% [1], it is sometimes referred to as “liquid protein” [2]. It has been estimated that the approximately 1,500,000 tons of porcine blood produced yearly in China has a protein content equivalent to that of 2,000,000 tons of meat or 2,500,000 tons of eggs [3]. Thus, a valuable protein source is lost if animal blood is discarded as waste and this is compounded by the resulting serious environmental pollution problems. Many countries require that animal blood be disposed off in an environmentally friendly manner, which is a capital intensive process. Accordingly, to eliminate a sizeable pollution hazard and prevent the loss of a valuable protein source, efforts have been made to ensure the utilization of animal blood on a massive scale. A further incentive is the increased profits to be made through adding value to the blood.

The environmental, nutritional and economic benefits derived from the maximal utilization of animal blood, coupled with recent advances in blood collection and processing techniques, have led to a myriad of blood protein ingredients becoming available for use in foods and dietary supplements to serve specific needs (Table 1). By 2001, it was estimated that the food industry was utilizing 30% of the blood produced in slaughterhouses [4]. Current utilization likely surpasses this figure, though there is no data to confirm this. Plasma, the liquid portion of blood remaining after the blood cells [white blood cells (WBCs), red blood cells (RBCs), and platelets] have been removed, is most widely used in the food industry because it is neutral in taste and devoid of the dark color associated with the red blood cells (and hence whole blood) [5]. The traditional use of whole blood and its separated red blood cells as ingredients in food products is generally restricted to such products as blood sausages where the black color is both expected and acceptable [2]. The cellular portion of blood (and for that matter whole blood) has not enjoyed widespread usage in the food industry as a result of its heme component, which imparts an undesirable color, odor and metallic taste to the final product [6,7]. Even in trace quantities hemoglobin imparts a dark-brown color to foods [8]. Their use is also restricted because the cellular fraction is thought to have a higher microbial load, although this has been disproved [9]. The amount of cellular fraction-derived products used in food has traditionally been limited to 0.5 to 2% of the product in order not to jeopardize the sensory qualities of the final

Table 1. Examples of blood-derived protein ingredients used as food additives and dietary supplements

Product	Company	Source of blood	Description
Fibrimex®	Sonac BV, Netherlands	Porcine or bovine	Thrombin and fibrinogen protein isolate
Plasma Powder FG	Sonac BV, Netherlands	Porcine or bovine	Plasma with increased fibrinogen concentration
Harimix (C, P or P+)	Sonac BV, Netherlands	Porcine or bovine	Stabilized hemoglobin
Hemoglobin	Sonac BV, Netherlands	Porcine or bovine	Frozen or powder hemoglobin
PP	Sonac BV, Netherlands	Porcine or bovine	Frozen or powder plasma
Prolican 70	Lican Functional Protein Source, Chile	Bovine	Spray-dried bovine plasma concentrate
Prietin	Lican Functional Protein Source, Chile	Porcine	Spray-dried porcine whole blood
Myored	Lican Functional Protein Source, Chile	Porcine or bovine	Natural colorant obtained from the red pigments of blood
ImmunoLin®	Proliant, USA	Bovine	Bovine serum concentrate
B7301	Proliant, USA	Bovine	Spray-dried bovine red blood cells
AproRed	Proliant, USA	Porcine	Stabilized hemoglobin
Aprofer 1000®	APC Europe, Spain	Porcine or bovine	Heme iron polypeptide
Proferrin®	Colorado Biolabs Inc., USA	Bovine	Heme iron polypeptide
Vepro 95 HV	Veos NV, Belgium	Bovine	Globin (hemoglobin with the heme group removed)
Plasma	Veos NV, Belgium	Bovine or porcine	Liquid, powder, frozen or flaked plasma

product [10]. This notwithstanding, recent efforts have focused on improving the utility of the cellular fraction in food proteins by removing the heme component using various techniques [6, 11-16] to produce an off-white product known in the food industry as globin or decolorized blood. Many of these treatments, however, are either not cost-effective, impart a salty or bitter taste to the globin recovered [17], or are only partially successful in alleviating the dark color associated with the heme component [8]. Thus, further processing methods continue to be devised to produce globin that lacks the bitter taste and has a whiter appearance. One such method involves treating RBCs recovered from blood with papain to release the heme, followed by further treatment with a bleaching agent, sodium hypochlorite. The isolated globin is almost tasteless with a white color [17].

Despite the technical successes achieved, animal blood proteins remain underutilized, largely due to consumer concerns. This paper reviews some of the specific uses of blood proteins in both the meat and non-meat industries and discusses the consumer issues hindering its maximal utilization.

2. Meat industry

The meat industry uses the bulk of the blood proteins employed as ingredients in the food industry, mainly as a binder but also as natural color enhancers, emulsifiers, fat replacers and meat curing agents. These will be discussed in turn below. However, some limited usages, for example as biodegradable natural casings for sausage products, will not be considered as such blood-based films have a relatively high solubility which may limit their application [18].

2.1 Binder

Binders have traditionally been used in meat products to counter the textural and sensorial changes brought about by processing. In addition to absorbing the moisture that is released from meat during thermal processing, they are used to bind water and fat to stabilize meat emulsions in ground meat products [19]. The development of comminuted or emulsion type meat systems that incorporate binders allows more efficient utilization of tough meat from spent animals. The partial replacement of meat with binders reduces product costs while improving, or at least maintaining, the nutritional and organoleptic qualities of the final product [20]. Binders have a macromolecular structure that has the capacity to form matrices that retain aroma and nutrients and also entrap large amounts of water in such a manner that exudation is prevented [21]. They are also used in the restructuring of meat, which is geared towards the transformation of lower value cuts and quality trimmings into consumer-ready products of higher value that resemble intact muscles such as steaks, chops or roasts. Meat restructuring is also driven by increasing consumer demand for exact portions in commercial meat and fish products.

Blood plasma and isolated blood proteins are both used as binders in the meat industry. Plasma functions as a binder in meat systems due to its ability to form gels upon heating, while the performances of plasma proteins are comparable, if not superior, to other binders. In terms of binding properties, blood plasma may be an alternative to the egg albumen traditionally used in the food industry for binding purposes [22], and remains the standard by which to judge other binders. Interestingly, plasma powders have been reported to be

less effective than egg white powder, though still better than other binders (meat powders, gelatin, wheat gluten, isolated soy protein, and a combination of sodium alginate and calcium carbonate [23]). However, this study pointed out that the superiority of egg white powder as a binder over plasma powder was based solely on muscle to muscle binding; accurate muscle food product binding should consider not only muscle-to-muscle binding but also muscle-to-fat and fat-to-fat binding and plasma powders exhibited higher muscle to fat and fat to fat binding compared to egg white powder [23]. At acid pH, plasma produces soft and exuding gels and so is not effective as a gelling agent or capable of water retention in products with a low pH such as fermented products, as these functional properties are strongly affected by changes in the protein structure brought about by the acidic environment [24]. Subjecting porcine plasma to simultaneous treatment with microbial transglutaminase and high hydrostatic pressure has been shown to improve the gelling properties of porcine plasma under acidic conditions [25].

Besides their excellent functionality as binders, the utilization of blood proteins in the preparation of restructured meats offers a number of health and industry benefits. In the past, preparation of restructured muscle products required tumbling meat pieces with salt to extract soluble protein for meat binding. This tumbling action, however, damages the texture of the product and the use of salt is also a concern for consumers who are aware of the link between the sodium content of food and the increased risk of cardiovascular and bone disease [26, 27]. Conventionally, the process also involved the use of heat to bind the myofibrillar proteins extracted from the meat by the combined salt and tumbling action, and as such was referred to as hot-set binding [28]. The problem with the hot-set method is that the product must be sold either pre-cooked or frozen because product binding is very low in the raw state. A cold set binding agent that relies on the physiological clot forming action of the plasma proteins fibrinogen and thrombin is available commercially as Fibrimex®, and can be obtained from both bovine and porcine sources. Fibrimex® is produced through a patented process by the Dutch company Sonac BV and its binding action is based on the transformation of fibrinogen into fibrin by the action of thrombin. The fibrin produced then interacts with collagen, enabling the binding of meat pieces in reconstituted meat [29]. Fibrimex® produces restructured meat that can be sold in the chilled or raw state, with an eating quality comparable to that of cuts from intact muscles. The cold-set method utilized reduces oxidative rancidity [30] and discoloration [31], both of which are common with the hot-set method. A further advantage is that it develops sufficient bonds to eliminate the cavities [29] invariably created in the meat pieces during deboning. Products made with this cold-set binder are more versatile, as they can be treated and cooked in ways similar to fresh meat. Sonac BV also produces a cold set binder known as Plasma Powder FG, a plasma product with an increased fibrinogen concentration.

Two other commonly used cold set binders in the industry are Activa™ TG-RM (a transglutaminase obtained from microbial sources) and alginate (a polysaccharide that works in combination with a divalent cation). Compared to Activa™ TG-RM, Fibrimex® has been reported to produce less binding in both raw and cooked restructured pork [32]. Several studies have reported that restructured beef steaks formulated with Fibrimex® produced a weaker bind compared with alginate. However, once cooked, Fibrimex® steaks were found to have a binding strength equal or stronger than that of steaks restructured with alginate [33, 34]. Interestingly, other researchers have reported the binding strength of both raw and cooked steaks formulated with Fibrimex® to be stronger than those

reformulated with alginate [35]. In this study, whereas some of the sensory panelists scored alginate formulated meat samples as bland or lacking uniformity in texture, no such unfavorable comments were made regarding the Activa™ and Fibrimex™ samples. Nevertheless, all three binders were rated as producing satisfactory binding in re-formed steaks and having overall acceptability [35]. These perceived differences may arise as a result of differences in the experimental procedure, for example the type of muscle used and the amount and proportion of binder used. More research is needed to identify the optimum amount of plasma proteins to utilize in particular meat products to achieve the desired results.

2.2 Natural color enhancer

Color is known to be one of the main factors used by consumers when evaluating the quality and freshness of meat products. The trend towards natural colorants due to adverse reactions to some artificial food dyes (and their impurities) [36, 37] has resulted in the substitution of artificial colorants with natural ones where technologically feasible. Although there are many safe natural sources of colorants, their suitability for use in foodstuffs depends largely on the availability of the raw material. In this sense, hemoglobin, which is obtained from slaughtered animals, represents a good source of natural red colorant given the large quantities of blood generated daily. It is important to note that the use of hemoglobin as a colorant has the added benefit of combating iron deficiency, which will be discussed in detail in a later section of this paper. However, its red color is unstable and largely dependent on the oxidation state of the heme iron. Oxyhemoglobin, the dioxygen ferrous form is bright red in color but when the heme iron is oxidized to Fe^{3+} the resulting hemoprotein, known as methemoglobin (metHb), has a characteristic brown color that is undesirable. Fortunately, the concentration of metHb in mammalian red blood cells is typically only 1 to 3% of the total pigment, mainly as a result of the presence of metHb reductase [38]. However, the processing techniques utilized to preserve the shelf life and ensure the safety of the product, greatly increase the amount of metHb due to hemoglobin autooxidation [39]. Although spray-drying is a good way to preserve the red blood cell fraction, even better than freeze drying [39], and is a necessary step to ensure the microbial quality of blood-derived ingredients, oxidation of the hemoglobin may continue during the storage of the spray-dried powder. Because of the unstable nature of hemoglobin, its use as a food ingredient is only possible once it has been stabilized. Hence, hemoglobin powders that are used as food colors are treated with protecting agents that form complexes with hemoglobin, prevent oxygen accessibility to heme iron and ultimately reduce hemoglobin auto-oxidation. The following few paragraphs will look at some of the hemoglobin-stabilizing techniques employed to make hemoglobin more useful as a natural colorant in meat products.

Chelating agents such as nicotinic acid and nicotinamide that have the ability to form complexes with the heme moiety have been reported to be effective in preventing hemoglobin auto-oxidation during drying and subsequent storage [40]. Nicotinic acid at a concentration of 2% w/v has been found to considerably retard the deterioration of hemoglobin concentrate during both spray-drying and subsequent storage. A positive effect was also detected with 2.5% w/v of nicotinamide, though the results were not as impressive as for nicotinic acid. However, in the presence of starch gels nicotinamide proved better at stabilizing the red color of hemoglobin in comparison with nicotinic acid [39]. The use of these chelating agents is not without problems. Nicotinamide, for example, has been found

to be a harmful vasodilator [41]. Conversion of hemoglobin into the more stable and organoleptically acceptable carboxyhemoglobin by saturation with carbon monoxide (CO) has been proposed as an alternative method to stabilize hemoglobin. The underlying principle of the technique is based on the strong affinity between CO and hemoglobin [42] and the low dissociation of the complex [43] that provides a strong and enduring linkage of the CO to hemoglobin, which can thus tolerate cooking and/or dehydration. In addition, CO-treated blood has a redder color and hence greater potential for use in meat product formulations [44]. Studies have shown that CO-treated porcine spray-dried blood stored in low oxygen transmission rate (OTR) bags retained an acceptable reddish color after 12 weeks of storage compared to untreated dry blood, which turned brown immediately upon drying. Comparatively, CO-treated porcine spray-dried blood in high oxygen transmission rate (OTR) packaging presented color indices similar to those of untreated blood after 12 weeks of storage. The authors explained that the observed difference could be as a result of the oxygen permeability of the high OTR bags and light oxidation, which caused the otherwise stable carboxyhemoglobin to turn brown [45]. Liquid porcine blood treated with CO at different pH levels (7.4, 6.7, and 6.0) and kept under refrigeration for 4 days maintained a more stable and attractive red color than fresh blood. The study was restricted to 4 days of refrigerated storage as this is the typical period for which the bacterial count remains acceptably low and hence the blood maintains a safe microbiological profile, allowing its safe utilization as a food ingredient [44]. There are currently several such hemoglobin-containing products available for use as natural color enhancers in meat products. However, effective stabilizing methods for hemoglobin remain an issue. It is possible that shifting the focus to the development of better packaging technology may avoid the need to use stabilizing agents, resulting in the production of hemoglobin-based colorants that are both safe and more natural.

2.3 Emulsifier

Emulsifiers are utilized in emulsified meat products such as frankfurters, pate, luncheon meat and mortadella to bind meat proteins, fat and water in a stable emulsion. Emulsifiers also help redistribute the fat finely throughout the product so the right texture is obtained. Emulsifiers often substitute for fat in association with other ingredients in low-fat meat products to give the final product sensory qualities comparable to those of their full-fat versions. The food industry has access to a wide range of emulsifiers suitable for use in foods and, in particular, meat products though these tend to be proteins. Proteins are the most common emulsifying agents in the food industry because they occur naturally and are generally both non-toxic and widely available [46]. Proteins that are used as emulsifiers are obtained from a variety of sources, both animal and plant. Processors, however, generally prefer animal proteins because they are better emulsifiers than plant proteins. Amongst the emulsifiers obtained from animal sources, casein and its salt derivatives are the most popular and are widely used in the food industry for this purpose. Less expensive protein sources with comparable emulsifying capacities are being sought to replace casein, which is expensive because of its high processing costs [47]. This is particularly important in developing countries, where milk products as casein must be imported.

Blood proteins have been found to have emulsifying properties that are comparable, and even in some situations, superior to those of casein and can therefore adequately replace

casein as the emulsifying agent in meat products. Silva and others [47] compared the emulsifying properties of sodium caseinate (SC) and globin isolated either by the acidified acetone (AG) method or the carboxymethyl cellulose (CG) method in the absence and presence of salt. In the absence of salt AG and CG showed a higher emulsifying capacity (EC), which measures the ability of the emulsifier to migrate to the oil-water interface, than SC. When salt was present, however, SC showed a higher EC than globins. The emulsifying activity index (EAI), which measures the ability of the emulsifier to stay in the oil-water interface immediately after the emulsion has formed, was greatest for AG with salt present. In the absence of salt, both SC and AG exhibited EAIs that were greater than that of CG. At no and very low salt concentrations there was no difference in emulsion stability (ES) (a measure of the ability of the emulsifier to stay in the oil-water interface after storage for a while or heating of the emulsion) among all three proteins. ES was, however, greater for CA for a salt concentration of 0.25mol/L. Since the emulsifying property is a cooperative function of EC, EAI, and ES, it seems that casein as an emulsifier has an edge over globin in high salt products. This would, however, not be an advantage in the food industry, which is tending to minimize the use of salt in its products because of the problems associated with high salt consumption mentioned above.

Globin also compares favorably to other proteins used as emulsifiers in meat products and food products in general. Crenwelge and others [48] compared the emulsifying capacities of globin with those of cotton seed, soy and milk proteins and reported that globin had the best emulsifying capacity. The emulsifying activity of globin was greater (EAI of 28) than that of either hemoglobin or ovalbumin, with EAI values of 14 and 8, respectively. Other authors have also reported globin to have emulsifying activity superior to that of hemoglobin and ovalbumin, although BSA, which had an EAI value of 31, was higher. A combination of peptic hydrolysis and the addition of carboxymethyl cellulose (CMC) to globin successfully raised the emulsifying activity of globin to above that of bovine serum albumin (BSA) [49]. All the blood proteins (globin, BSA, hemoglobin) have been shown to be better emulsifiers than egg (ovalbumin), which is generally considered to have good emulsifying properties. However, plasma is reported to have better emulsifying capacity than globin when incorporated into sausages and it can be incorporated into meat products at higher inclusion levels than globin without jeopardizing the visual appeal of the product because the decolorized product retains a reddish hue that is transferred to the final product [50].

In summary, blood proteins have excellent emulsifying capacity and can adequately replace casein and egg in emulsified meat systems. Blood proteins such as hemoglobin, when used as emulsifiers, have the added advantage of providing a good source of heme iron. Compared to casein and egg, both of which are potent allergens and are thus among the "big eight" allergens covered under the Food Allergen Labeling and Consumer Protection Act (FALCPA) law, blood proteins do not present a problem of inducing allergic reactions. In situations involving highly salted products, however, plasma or globin that has undergone further processing such as a combination of peptic hydrolysis and CMC addition to enhance its emulsifying capacity are likely to be the best option.

2.4 Fat replacer

Fat plays an essential role in the diet as a source of vitamins, essential fatty acids and energy [51]. Besides its nutritional role, fat has a major effect on the tenderness, mouth feel, binding

properties, juiciness and overall appearance and palatability of processed meats [52]. On the other hand, empirical data has shown a correlation between dietary fat and cardio-vascular disease (CVD) and some types of cancers (colon, breast and prostate). Thus, for today's health conscious consumers, reducing dietary fat is a major consideration. The meat industry has been seriously affected by adverse publicity due to the high fat content and unhealthy fatty acid composition of their products, particularly those that include emulsified meat. For example, the minimum fat content in finely ground meat emulsion type products such as frankfurters, dry fermented sausages, and spreadable dry sausages are about 10%, 20-30%, and 20%, respectively [53] and this includes considerable amounts of saturated fatty acids. In response to consumer demand, the food industry has developed an assortment of low-fat meat products but given the important physico-chemical and sensory role that is played by fat, replacing fat in food products is a daunting task: if not properly done, the sensory appeal of the product will be severely jeopardized. Thus, low-fat meat varieties that are not acceptable in terms of taste and appearance will simply not sell, regardless of any health benefits [54].

Studies have shown blood proteins to have potential as fat replacers in meat products, contributing soluble proteins while at the same time reducing costs. They also reduce the caloric content of food for every gram of fat replaced, since proteins contain fewer calories than fat. In addition blood proteins are a natural product and hence in line with present day consumer demands for natural foodstuffs. In a study by Viana and others [55], replacing the fat with globin, plasma, and a combination of plasma and globin resulted in ham pates with 25%, 35% and 35% less fat, respectively. Among the treatments, the reduction in fat observed was not statistically different. The use of these fat replacers also resulted in an increase in moisture and protein content with no observed change in aroma, taste and consistency. The color of pate was, however, reduced in all the treatments containing blood proteins in comparison with control samples with no added blood protein. Reduction in color on addition of fat replacers is not peculiar to blood proteins as this occurrence has also been reported with the incorporation of other fat replacers in meat products by several authors, as cited by [56]. In short, fat influences the color of meat products and reducing its level will invariably affect the color attributes of the product.

The use of blood proteins as fat replacers compared favorably with other commercially available fat replacers, as shown by the results of several studies. Confrades and others [57] compared the effect of plasma and soy fiber as fat replacers in bologna sausage, concluding that plasma protein had a greater influence on the binding and textural properties of bologna than soy fiber and was hence a better choice. Desmond and others [58] evaluated the sensory, chemical and physical properties of low-fat beef burgers formulated with nineteen different commercially available fat replacers including Plasma Powder U70 and Protoplus® U70, both of which are blood-derived products. They reported that beef burgers formulated with these blood proteins had poor overall quality and flavor attributes. It is worth noting, however, that burgers containing these blood proteins had individual sensory traits that were superior to burgers containing other fat replacers. For example, the moistness and juiciness of burgers formulated with Protoplus® U70 was superior to 15 of the 17 non-blood derived fat replacers tested. Burgers containing Protoplus® U70 were rated as having the best overall texture, together with three other non-blood fat replacers. Inclusion of these blood proteins also produced physical characteristics that were superior to most of the other fat replacers used. For example, beef burger containing these blood

proteins had % cook yields that were greater than 13 of the 17 other fat replacers tested. Similarly, the WHC of beef burgers containing Plasma Powder U70 and Protoplus70 were superior to 15 and 12 other fat replacers, respectively.

Blood proteins therefore provide a cheaper alternative protein for use as fat replacers in the production of low-fat meat products. However, this may not satisfy some consumers due to the non-meaty flavor resulting from their inclusion. Guzman and others [59] have attributed the lower meaty scores when blood proteins are incorporated to the specific flavor characteristics contributed by each type of blood protein. Since a single ingredient is unlikely to produce the desired effect as a fat-replacer in the production of low-fat meat products [57], combining plasma proteins with other fat replacers with superior meaty flavor characteristics promises to be a worthwhile approach.

2.5 Curing agent

Nitrite plays a multifunctional role in the curing process of meat. It is responsible for the red color [60, 61], and characteristics and desirable flavor of cured meats [62, 63]. It may also influence the texture of the product through the promotion of protein cross-linking [64]. Furthermore, nitrite acts as an antimicrobial agent [65, 66]. Although nitrite continues to be the most widely used of all food additives, its use has come under scrutiny in recent years. This is because nitrite has a tendency to react with amines, amides, amino acids and related compounds present in the meat to produce carcinogenic N-nitroso compounds. In addition, ingested nitrite (in the form of residual nitrite) may react with various substances in the gastrointestinal tract (GIT) to produce these N-nitroso compounds [67, 68]. Various studies have confirmed the presence of volatile N-nitrosamines in cured meat compounds, though there are differences in both the type and the quantities reported by different authors [69]. To address these issues, efforts are being made to reduce the amount of nitrite used in the curing system or to develop alternative methods of curing that avoid the use of nitrite altogether. The latter approach may be the best option; the most attractive and reliable method for preventing the formation of N-nitroso compounds is the total elimination of nitrite from the curing process [70]. However, finding a viable alternative for nitrite in the meat curing process is problematic as it is extremely difficult to find a single compound that can fully reproduce the multifunctional role of nitrite described above. To date, no single agent has been identified that is capable of replacing nitrite. The best option may be the use of a composite mixture containing no nitrite but instead comprising a colorant, an antioxidant, and an antimicrobial agent, among others, to produce nitrite-free cured meats [71].

One of the colorants suggested for use in a composite nitrite-free cocktail for meat curing is cooked cured meat pigment (CCMP), which when added to meat prior to cooking duplicates the color typical of nitrite-cured meats. CCMP, a mono and/or di-nitric oxide hemochrome, is synthesized directly or indirectly through a hemin intermediate. The pigment is prepared by reacting bovine or porcine red blood cells with a nitrosating agent and at least one reducing agent at a suitable elevated temperature. The resultant product is either spray-dried or freeze-dried to obtain a powder. The pigment may also be treated with a starch and a binding agent (e.g. gum) to stabilize the pigment [72]. Addition of CCMP to comminuted pork meat at levels of 3 to 30mg/kg produced a pink color after heat-treatment that was visually similar to that of nitrite-treated pork [73]. Although the best performance of CCMP is in meat systems containing low to intermediate concentrations of myoglobin, its

performance in dark meats has been found to be satisfactory [71]. Results support CCMP as a healthy substitute for nitrite salt, as CCMP has been found not to be mutagenic compared to nitrite. The use of CCMP in meat emulsions therefore results in a drastic reduction in the residual nitrite that contributes to the formation of carcinogenic N-nitroso compounds in the GIT [69].

Though CCMP was initially developed for use in cured meat products to duplicate the characteristic red color associated with the use of nitrite, it has also been found to have antioxidant properties similar to those of nitrite [74]. At the time of writing, CCMP is not yet available commercially, possibly due to its unstable nature although this is being improved with the use of stabilizers. Further studies to include the addition of antimicrobials in the formulation of CCMP to duplicate all three major functions of nitrite in cured meats may make possible the commercialization of this product in the near future.

3. Non-meat usage

Although the meat industry remains the predominant user of blood proteins, other sectors of the food industry also utilize them in the production of such items as baked goods, dietary supplements and functional foods, usually as egg replacers, protein supplements, iron supplements or a source of bioactive compounds. Some of these uses will be reviewed in this section.

3.1 Egg replacer

In baked goods such as cakes, egg proteins play an important role in defining such final product characteristics as cake volume and texture due to the unique foaming, emulsifying, and heat coagulation properties of eggs [75]. Among the various ingredients utilized in baked goods, eggs are the most costly [76] and they are also a significant source of cholesterol. The use of alternative ingredients to partially or totally replace eggs in baked goods is therefore of interest to processors not only to reduce cost but to provide lower cholesterol baked goods varieties. Blood proteins have been found to serve as useful egg replacers, as reported by several authors. Spray-dried plasma protein concentrates marketed for diverse use in food processing, including as egg replacers, are sold at a cost of only about one-third that of spray-dried egg whites [76].

Lee and others [76] evaluated the sensory and physical properties of white layer cakes made by substituting egg (as dried egg white) either partially (25%, 50% and 70%) or totally (100%) with spray-dried plasma. The authors reported that cake volume was not affected by egg replacement, although cake symmetry decreased at 75% and 100% substitution. In terms of shrinkage, cakes formulated using partial replacement had lower shrinkage values than control cakes made entirely with dried egg white; those formulated with total replacement of egg with plasma produced shrinkage that did not differ significantly from the control cakes. This suggests that so far as critical quality indices such as volume and shrinkage are involved, replacing egg with plasma produced superior products, particularly at partial replacement. It is worth noting that the authors reported that replacing egg with plasma at all levels resulted in products with darker and tanner crust and crumb, but these differences notwithstanding, trained panels liked the control cakes and those prepared with partial or total replacement of egg with plasma equally. The same study [76] utilized a blend of 90%

enzymatically hydrolyzed plasma and 10% beef stock as a replacement for eggs in the formulation of devil's food cake, a whole-egg product. The results are not relevant here, however, due to the inclusion of beef stock which makes it difficult to deduce which product effects can be ascribed to the inclusion of plasma only. In a similar study, Johnson and others [77] investigated the feasibility of replacing egg white and whole egg with freeze-dried plasma in the manufacture of white and yellow layer cakes, respectively. In the latter case, 1.3% lecithin was added to compensate for the natural phospholipid content of whole egg. The control cakes consisted of 14% egg white solids and 3% added salt, whereas the experimental cakes contained 18.6% plasma solids with no added salt. The formulation was selected to ensure equivalent protein and salt concentrations for the plasma and egg-white control cakes. The authors reported that cakes made with plasma were at least equal if not greater in volume and also shrank less than the egg-white control cakes. In the case of the yellow layer cakes, the volumes of those made with plasma solids was between 7 and 15% less than those made with whole egg at equivalent protein levels. They concluded that blood plasma was effective in replacing all the egg white or whole egg in cakes and hence shows potential as a low cost egg substitute for use by the baking industry.

Decolorized bovine blood has also been considered as a replacement for egg white in white layer cakes. Although cakes made with decolorized blood had volumes comparable to those made with egg white, cakes made with decolorized blood had an objectionable flavor [78]. The poor sensory quality of cakes formulated with decolorized blood may be due to residual heme. Raeker and Johnson [79] also compared the baking properties of egg white and plasma and their component proteins, reporting that cakes made with egg whites had slightly greater volumes than their counterparts made with freeze or spray-dried plasma, though the differences were not significant. Among the blood proteins, only γ -globulin had baking properties superior to those of plasma. They also noted that separating fibrinogen (which by itself produced the smallest cake volume among the blood plasma proteins) from plasma increased cake volumes. However, given that the primary purpose of using blood proteins as a substitute for eggs in baked goods is to reduce costs, the additional expense incurred by fractionating plasma into its component proteins appears counterproductive and there appears to be no incentive for using fractionated plasma proteins for this purpose.

In summary, plasma proteins can be very effective low cost alternatives for replacing eggs in baked goods. The use of blood proteins derived from the cellular fraction, as decolorized bovine blood leads to the production of baked goods with objectionable odor, however, and further research is needed to counter the flavor defect associated with its use. Also, since the main objective of replacing eggs with plasma is to reduce cost, the use of expensive fractionated blood proteins for this purpose cannot be justified. The removal of fibrinogen from plasma, leading to an increase in cake volumes, may be a practicable means of improving the efficiency of plasma for this purpose, as separation of this component from plasma by centrifugation would be relatively inexpensive.

3.2 Protein supplement

Protein malnutrition is a major problem in developing countries during the transitional phase of weaning in infants, retarding their physical and mental development. Introducing weaning foods containing the right quality and quantity of protein during this transitional period has been recommended by international bodies such as the WHO and FAO as a

necessary preventive measure [80]. In many developing countries, diets that are used to supplement breast feeding during the transition from exclusive breast feeding to a mixed diet (between the ages of 6 to 8 months) and thereafter as a major breakfast meal (between age 1 to 6 years) are usually produced from maize flour [81, 82]. Kwashiorkor, a severe protein deficiency disease that is common in some parts of the world has been linked to the quality and quantity of protein in maize when it is the sole source of protein for infants [83]. Unfortunately, animal proteins such as milk and meat that contain high quality proteins in good quantities tend to be expensive and not readily available in developing countries. Consequently, concerted efforts by researchers, government and international bodies to eliminate protein malnutrition have generally been directed towards the use of plant proteins (e.g. from legumes and soy) as substitutes for animal proteins to supplement grains and cereals in the formulation of weaning diets. However, these plant proteins have lower protein levels, lack essential amino acids, and/or contain antinutritive factors [84-86]. Processing plant proteins such as soy to eliminate their antinutritive factors greatly increases the cost and hence defeats the purpose of their use as a low-cost high quality protein to substitute for animal proteins. The extreme processes involved also reduce the protein quality of the final product due to denaturation [87]. Studies with newly weaned pigs have established that incorporating blood proteins into their feeds accelerates daily weight gains [88-89]. Similar results have been observed in newly weaned mice [90], indicating that the benefits of including blood proteins in the diet is not species-specific. Thus, blood proteins, which are abundant, cheap, readily available, devoid of antinutrients, have a good amino acid profile, and a proven track record in animal nutrition, have been suggested for use as protein supplements in infant formula to tackle protein malnutrition. Several studies that have investigated the feasibility of using blood proteins as protein supplements to combat protein malnutrition in developing countries are highlighted below.

Begin and others [91] compared the effect of supplementing a maize diet with bovine serum concentrate (BSC) alone or in combination with multiple micronutrients (MMN), or with whey protein concentrate alone or with MMN (as the control) on the physical growth and micronutrient status of 132 Guatemalan infants between the ages of 6 to 8 months who completed the 8-month long study. Over the period of the study, the subjects gained about 1.8kg in weight and about 8cm in length, which corresponds to approximately 63% and 75% of the expected weight and length gain, respectively, for their age counterparts in North America. The increase in growth by treatment group was not, however, significant. So far as micronutrient status was concerned, the authors reported that infants who received a combination of WPC and MMN showed higher final serum ferritin concentrations than those receiving a combination of BSC and MMN. All other markers of micronutrient status were the same. Their conclusion was that supplementation with BSC or with BSC and MMN was not effective in preventing growth stunting of young children in a peri-urban setting in Guatemala, although they did not specify the basis for this conclusion. It seems likely that their conclusions were based on a comparison with North American children, which is not a good yardstick as growth charts in these regions differ from those used by the WHO. They did, however, note that the children who received the supplements containing bovine serum concentrate consumed about 12% less of the amount administered in comparison to those who received diets containing WPC. This suggests that the flavor of BSC was less popular with the children. It is possible that if more of the maize diet supplemented with BSC had been consumed by the children, the growth rate recorded for this group may have surpassed

that of the control group consuming whey supplements. Nonetheless, the results of this study demonstrate that blood proteins can adequately replace other animal proteins such as whey.

The adequacy of blood proteins as protein supplements has also been demonstrated by Oshodi and others [92] who investigated in vitro protein digestibility (using a multienzyme digesting system comprising trypsin, chymotrypsin, and peptidase), amino acid profile and available iron of an infant weaning food prepared from maize flour and bovine blood. Their results indicated an improved digestibility when blood protein concentrate was added to the maize. The amino acid profile of the formula was also satisfactory in meeting the essential amino acid requirement for infants as specified by the FAO/WHO joint committee [80]. The iron content of the formula was double that of maize alone. In this study [92], the bovine blood powder concentrate used was so bland in taste that its addition to the maize flour did not alter the overall flavor. This contrasts with the study discussed earlier where the blends containing BSC appeared to be less liked than those containing WPC, which is understandable as the subjects were still suckling and therefore not accustomed to unusual tastes (in this case BSC; WPC is similar to milk). In a related study by Lembcke and others [93], older children (between the ages of 9 and 25 months) admitted for rehabilitation for severe cases of protein deficiency (manifested as marasmus and kwashiorkor) were fed diets in which 25% or 50% of the dietary protein normally provided by milk was substituted with an equivalent protein from spray-dried bovine serum. Here, the formulas containing the spray-dried bovine serum were well accepted by the children. Also, weight gain on the test diets was no different from those on the control diet. It is important to note, however, that in this study [93] the milk fat in the spray-dried bovine serum diet was partially replaced by vegetable oil. The addition of fat, which plays a critical role in the organoleptic quality of foods, could also be responsible for the greater acceptability of the product in this case.

The studies outlined above support the use of bovine blood proteins as efficient protein supplements in cereal and grain based weaning and infant diets as a measure to tackle protein malnutrition problems in developing countries. However, if the target consumers are infants that are starting to consume solid foods, the addition of vegetable oil or other flavors may be necessary to improve the palatability and consequent acceptability of formulas. Where anemic children are involved, the use of bovine blood instead of plasma or serum offers a useful way to combat this condition owing to the heme content of blood.

3.3 Iron supplement

Iron deficiency is the most common nutritional deficiency in the world affecting about 20% of the world's population, with women and children at greater risk [94]. It is induced in part by plant-based diets that typically contain non-heme iron, a form of iron that is poorly absorbed [95, 96]. In infants, iron deficiency can delay normal infant motor and mental function [97, 98]. Iron deficiency manifesting as anemia in pregnant women increases the risk of small or preterm babies [99], which are more likely to suffer from health problems or die in the first year of life. It can also cause fatigue in adults, impairing their ability to do physical work [100]. Several strategies are commonly directed at combating iron deficiency, including supplementation with capsules and tablets and fortification of processed foods. However, in developing countries, food-based strategies remain the most sustainable approach for addressing iron and other micronutrient deficiencies [101]. Fortifying staple foods with heme-iron, which is better absorbed than non-heme iron because its absorption is

essentially unaffected by other dietary factors [102], has therefore been suggested as a measure to overcome the problem of iron deficiency. Heme iron is known to result in less gastrointestinal discomfort and oxidative stress than non-heme iron [103, 104]. Dietary sources of heme iron are only of animal origin such as meat and liver, which is often a problem in developing countries where animal products are expensive and not readily available. As bovine blood has the largest amount of heme iron than any animal source [105] and is readily available, it has therefore been suggested for the fortification of staple foods. The success of blood-fortified foods in addressing iron deficiency has been reported by several researchers.

Kikafunda and Sserumaga [105] investigated the physical, chemical, microbiological and shelf life characteristics of bovine blood powder and the sensory attributes of a bean sauce fortified with it and found that the fortified bean sauce was less liked than the non-fortified counterpart in terms of all the sensory attributes (color, flavor and overall acceptability) considered. This is understandable, as the presence of the heme moiety generally imparts an objectionable color and flavor to the final product. It is worth noting, however, that although the fortified bean sauce was less liked it was not rejected outright. As children are among the most vulnerable groups affected, the introduction of heme iron fortified foods into school lunch programs becomes a viable way to combat this problem. The suitability of such an approach has been demonstrated by Walter and others [106], who investigated the effect of bovine-hemoglobin-fortified cookies on the iron status of school children in a nationwide school lunch program in Chile. Average serum ferritin values in both boys and girls were greater in the heme-fortified cookie. In addition, the fortified cookie group had a remarkably low prevalence of anemia. These benefits were achieved at virtually no cost as the researchers estimated that the additional expense of fortifying the cookies increased the cost by only \$0.53 per child per year. As far as taste was concerned, the fortified cookies were virtually indistinguishable from the non-fortified cookies. It is possible that the different ingredients utilized in the formulation of the hemoglobin-fortified cookies may have masked the characteristic flavor of hemoglobin, rendering it imperceptible in this case. Some skeptics have opined that it is impractical to use hemoglobin as an iron supplement because it is low in iron (0.35%) [107], poorly soluble at low gastric pH [107], and its absorption is usually less than iron absorption from muscle [108, 109]. Heme iron polypeptide (HIP), a soluble heme moiety with an attached polypeptide obtained from the enzymatic digestion of bovine or porcine hemoglobin has been developed to overcome these concerns. The hydrolysis action enhances iron absorption by preventing the formation of large insoluble heme polymers [107]. Examples of commercially available HIP products are Proferrin® (Colorado Biolabs Inc., Frederick, CO) and Aprofer 1000® (APC Europe, S.A., Barcelona, Spain). The effectiveness of HIP as an iron supplement has been demonstrated by several researchers.

Quintero-Gutierrez and others [110] evaluated the bioavailability of a heme iron concentrate product incorporated into a chocolate flavor biscuit filling in sandwich-type biscuits using the pig as a human model. The heme iron was prepared by isolating hemoglobin from porcine blood followed by enzyme hydrolysis and subsequent separation of globin from the heme group using ultrafiltration. Twenty pigs with slightly induced iron deficiency at weaning were divided into the study group (fed a low iron diet [452.7mg/kg] and sandwich biscuits with the heme iron fortified chocolate flavor filling) and the control group (fed normal food containing 537.1mg/kg of ferrous sulphate). The bioavailability of heme iron in the study group was 23% greater than that of the control group despite the fact that the

control group consumed greater amounts of iron. The study group also exhibited better weight increase and reduced mortality compared to the control group. The product had a good sensory and functional appeal as it had a chocolate color and smell, creamy appearance, and appropriate spreadability. The same research group went on to evaluate the acceptability and bioavailability of the heme iron enriched chocolate flavor biscuit filling in sandwich-type biscuits in adolescent girls living in a rural area of Mexico [111]. The girls were divided into three groups: the placebo control (PC) group (consisting of girls with the highest hemoglobin levels at baseline, who were given non-fortified biscuits), and two study groups one of which was given an iron sulfate fortified biscuit and the other a heme iron fortified biscuit. The iron content for both types of fortified biscuits was the same. The two study groups included both anemic and non-anemic girls. The researchers observed that iron bioavailability in the heme fortified biscuit group was 23.7% higher than that in the iron sulfate fortified biscuit group, indicating that the heme fortification was well absorbed and tolerated. At the end of the study, serum ferritin concentrations, a measure of the amount of iron stored in the body, were reduced in the control group and the iron sulfate fortified biscuit group, but not in the heme iron fortified biscuit group. Other studies have confirmed that supplementing with HIP produces a better iron status in terms of better iron absorption and higher storage (serum ferritin levels) compared to iron salts [112, 113].

The findings of these studies agree that hemoglobin provides a low-cost heme-iron source that can conveniently be used to fortify commonly consumed staples in order to address problems associated with iron deficiency. However, though enzymatic hydrolysis increases the iron absorption, the process will add to the cost and may not be suitable in all developing countries. Other ways to increase the iron absorption of hemoglobin without adding to the cost therefore requires further study. Also, in certain situations the addition of flavor components may be desirable to make the final product more acceptable.

3.4 Bioactive compounds

Functional foods have begun to attract a lot of attention because of the growing belief that foods should possess health-promoting qualities. Recent physiological and biochemical research has shown that the protein in food not only furnishes amino acids but also provides bioactive peptides after digestion or food processing [114]. Consequently, bioactive peptides produced from both animal and plant sources are now being widely investigated and have been reported to have antibacterial [115, 116], opioid [117], antitumor [118], antioxidant [119, 120], and angiotensin I-converting enzyme (ACE) inhibitory (anti-hypertensive) [121, 122] activities. Bioactive peptides derived from food proteins are considered milder and safer than synthetic drugs and are easily absorbed [123]. Non-conventional food sources are also being investigated; animal blood, which is both abundant and readily available but greatly underutilized as a protein source, is therefore being actively studied as a potential source of bioactive peptides.

Present day dietary habits have led to an increase in the prevalence of cardiovascular diseases, as evidenced by the fact that hypertension-related diseases make up half of the most common causes of death [124]. The renin-angiotensin-aldosterone system controls blood pressure and ACE plays a critical role by converting the non-active angiotensin I in blood into its active form, angiotensin II through hydrolysis. This, in turn, causes blood vessels to contract and blood pressure to rise. In addition, ACE deactivates bradykinin, a

nonapeptide that causes blood vessels to extend, by removing amino acids from its C-terminus, contributing to the rise in blood pressure. Thus, repressing ACE activity with ACE inhibitors lowers blood pressure [125]. However, ACE inhibitor drugs such as captopril, enalapril, alacepril and lisinopril that are utilized as anti-hypertensive drugs have a number of side effects such as cough, taste disturbance and skin rashes [126]. Accordingly, natural food-based anti-hypertensive alternatives may offer a valuable alternative.

Wei and Chiang [127] investigated the possibility of hydrolyzing porcine blood proteins using an enzyme mixture in a membrane reactor for the production of bioactive peptides. Red blood cells, plasma and defibrinated blood isolated from porcine blood were used as the substrates for hydrolysis. Of the three fractions, the red blood cells had the highest ACE inhibitor and antioxidant activities. The unpleasant dark red color of blood was also lost during the process, resulting in the production of a pleasant golden yellow colored product and rendering it more useful in the production of anti-hypertensive functional foods. Yu and others [123] also described the isolation and characterization of ACE inhibitor peptides from porcine hemoglobin. Three enzymes (papain, trypsin and pepsin) were used in the digestion of the globin isolated from hemoglobin and the most active hydrolysates from the peptic digestion identified. The peptides exhibiting ACE inhibitor activity were identified as LGFPTTKTYFPHF and VVYPWT, with the former been identified as a novel peptide. The two ACE-inhibitor peptides both competitively inhibited ACE and maintained the inhibitory activity even after incubation with GIT proteases. There is now a protein supplement product, Rifle High Powered Protein Powder, Chocolate (Theta Brothers Sports Nutrition Inc., Brick, NJ) on the market for body builders that contains a patented bioactive peptide from hemoglobin hydrolysate designated as VVYP. Although the manufacturers do not mention the role of this peptide in the formula, its inclusion is probably designed to offset any rise in blood pressure brought about by intense body building exercises. The ACE inhibitor activity of blood seems not to be species specific, as in addition to its demonstrated effect in porcine and bovine blood it has also been demonstrated in chicken blood hydrolysate [128], where of the three enzymes used (Alcalase, Prozyme 6, and Protease N), the alcalase digested chicken blood produced the highest ACE-inhibition activities.

According to the CDC (Centers for Disease Control), three pathogens (*Salmonella*, *Listeria*, and *Toxoplasma*), are responsible for 1,500 deaths each year in the US, representing more than 75% of those caused by known pathogens. Given the current context of food safety, protection utilizing natural products as preservatives could be beneficial for safe storage and distribution of meat products [129], which are often responsible for outbreaks of food-borne diseases. This is particularly important as some of the proposed means of addressing food-borne diseases such as irradiation are unacceptable for many consumers, who are increasingly demanding natural products. Blood is known to contain important elements (antibodies and leukocytes) that fight against infection. Turning to blood proteins as sources of anti-bacterial peptides is, therefore, justified and a number of researchers have investigated the anti-bacterial properties of peptides isolated from animal blood. Nedjar-Arroume and others [129] identified four antibacterial peptides in bovine hemoglobin. The total hemoglobin isolate obtained by peptic hydrolysis was found to inhibit the microorganisms *Micrococcus luteus*, *Listeria innocua*, *Escherichia coli*, and *Salmonella enteritidis*. Antibacterial activity towards the latter two organisms is of particular importance, as both organisms are frequently implicated in food-borne illnesses. Froidevaux and others [130] have also demonstrated the anti-bacterial activity of pepsin-digested bovine hemoglobin. The antibacterial peptide in

this case was the 1-23 fragment (VLSAADKGNVKA AWGKVGGHAAE) of the alpha chain of bovine hemoglobin and it exhibited antibacterial activity towards *Micrococcus luteus*, which is a bacterial strain commonly used as a sensitive strain for the detection of antibiotic substances. They did not, however, test the efficacy of the isolated peptide against common food borne pathogens, which is important because antibacterial activity against *Micrococcus luteus* does not necessarily imply antibacterial activity towards other organisms, as was pointed out by Nedjar-Arroume and others [29].

Lipid peroxidation is a serious concern for food manufacturers because it results in the production of undesirable off-flavors and potentially toxic reaction products. Oxidation of membranes and lipoproteins in the human circulatory system has been identified as the culprit in the pathogenesis of vascular diseases such as atherosclerosis and hypertension [131]. Many synthetic antioxidants, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate, are commonly utilized in food formulations to inhibit lipid peroxidation. However, the use of these synthetic antioxidants is strictly regulated because they represent a potential health hazard. The antioxidant activity of blood protein peptides has therefore been studied and demonstrated to offer an effective natural alternative to synthetic antioxidants. Xu and others [132] investigated the antioxidant activity of porcine plasma hydrolysate obtained by pepsin and papain digestion in a peroxidation system of aqueous linoleic acid. Both the pepsin and papain digested hydrolysates exhibited significant activities against linoleic acid oxidation and good DPPH free radical scavenging ability. However, the chelating power of the pepsin-digested hydrolysate was greater than that of the papain-digested hydrolysate. Their recommendation was therefore that pepsin digested porcine plasma hydrolysate has the potential to serve as a potent natural antioxidant in foodstuffs.

Albumin, a major protein in blood [133] is considered a major circulating antioxidant in the blood and Bishop and others [134] have reported that albumin protects cultured cells from oxygen radical damage. An in vitro study has also shown that albumin protects human low-density lipoproteins from oxidation [135]. Wang and others [136] therefore sought to examine the antioxidant ability of crude plasma and globulin hydrolysates and also that of the peptide fractions. Of these, the peptide fractions were found to have better lipid peroxidation inhibitory activity than vitamin E, a known antioxidant, while the antioxidant properties of both the crude hydrolysates and small molecular weight peptide fractions of albumin were superior to those of globulin. The authors noted that the hydrolyzation process was both easy and economical, and hence amenable to large-scale production.

Blood clearly has the potential to serve as a useful source of valuable peptides with anti-hypertensive, antioxidant, and anti-bacterial properties, among others. Several bioactive peptides that offer other health benefits such as analgesic and antinociception (reduction in pain sensitivity) activities have also been isolated from blood proteins; the review article by Gomes and others [137] provides more detailed information on some of these peptides that have been isolated from blood. However, there is still work to be done in the area of in vivo studies, as most of these demonstrated activities of blood peptides have been in vitro.

4. Consumer concerns

Despite the immense efforts devoted to ensuring that blood proteins are maximally utilized in the food sector, consumer concerns related to religious prohibitions, the belief that these

blood proteins are unsafe, the possibility of their fraudulent usage, and ethical issues prevent the realization of this objective. Interestingly, the available scientific data suggests that some of these concerns are actually groundless, as will be explained below. However, the anxiety expressed by consumers is real and warrants a concerted effort by government, processors and researchers to allay these fears and hence ensure the maximal utilization of blood proteins.

4.1 Fraudulent usage

The largest user of blood proteins in food is the meat industry. The economic advantage to be derived from their fraudulent use makes this a significant concern. Partial replacement of lean meat with blood plasma content offers a major economic incentive to the manufacturer, as the addition of 2% of blood plasma to a meat product can boost yield by 4 to 5% and substitute for up to 10% of the lean meat content [138]. It is therefore tempting for rogue manufacturers and food service providers to fraudulently utilize these blood proteins for economic gain. A typical case is the use of Fibrimex® as a meat binder, discussed earlier in Section 2.1. In May 2010, the EU voted to ban the use of Fibrimex® as the EU believes the product has no proven benefit and its usage carries an unacceptably high risk of misleading consumers. The concern was that Fibrimex®-reconstituted meat products would find their way into meat dishes served in restaurants, given the higher prices that can be obtained for pieces of meat sold as a single meat product. Legislators considered that consumers should be able to trust that the meat they are buying is a real steak and not simply pieces of meat glued together. The ban, however, never took effect as in accordance with Commission directive 2010/67/EU, Fibrimex® is permitted for use as a food additive for reconstituting food. In the US, where Fibrimex® is permitted at usage levels up to 10% [139] doubts about its usage such as those expressed in the EU have not been an issue. Interestingly, at no point did other products such as Activa™ TG-RM and alginate used for the same purpose as Fibrimex® come under scrutiny in the EU regarding their potential for fraudulent use. The same is true for Plasma FG, which is also derived from blood and produced by the same company for the same purpose. Such inconsistencies in regulations lead consumers to doubt the quality of these blood proteins. Efforts to ensure that their usage is not abused for economic gain at the restaurant level are, however, necessary as the problem has not yet been properly addressed. The development of effective methods for monitoring their presence in food products, along with frequent restaurant auditing by the appropriate authorities, is necessary to address this concern.

4.2 Religion and ethical reasons

Certain individuals, for example Jews and Muslims, do not consume blood because of the religious dictates enshrined in the Kosher and Halal dietary laws, respectively. Whereas some of these dietary laws concerning certain foods are not explicit and are therefore subject to individual interpretation, both codes unequivocally forbid the consumption of blood. In most parts of the world, particularly in the developing countries, animal slaughter is almost exclusively performed by Muslims, who condemn the use of blood as a food item. This is probably one of the main reasons why blood consumption by humans globally is very low [105].

Others such as vegans avoid consuming products of animal origin for ethical reasons. It is therefore imperative that appropriate labeling laws compelling manufacturers to declare the

presence of these blood proteins in simple and understandable layman's terms (as is the case with allergen labeling), are enforced to help the above-mentioned group of individuals to make the right food choices. In this respect, whilst many of the labeling laws are explicit and adequate, others may have to be revised. For example, beef blood is an acceptable ingredient for beef patties provided the product name is qualified as "Beef and Blood Patties" or "Beef Patties with Blood" [139]. Clear and explicit labeling is extremely helpful in protecting the interests of individuals who must avoid consuming food containing blood. This is not always the case, however; the permitted usage of Fibrimex® (as thrombin and fibrinogen) is contingent on the conspicuous display of the terms "Beef Fibrinogen and Thrombin", "Beef Fibrin" or "Fibrin" as product name qualifiers on the label [139]. Such technical labeling provisions are unhelpful and may have to be revised.

The situation is even worse when these blood proteins are used as ingredients in dietary supplements. Several of these dietary supplements have brand names on the label that include no indication that they are blood-derived. For example, Proferrin® lists no explicit information on the label that indicates HIP is derived from bovine hemoglobin (and hence from bovine blood). The only information given as to the source of the heme is that "Heme is a natural form of iron derived from animal sources and is intended for individuals seeking to maintain normal iron levels." A similar issue pertains to some of the dietary supplements on the market that contain Immunolin®; while some products such as Immune Advantage (Now Foods, Bloomington, IL) clearly state on the label that Immunolin® is derived from bovine serum, other products such as Daily Immune Defense (Distributed by Doctor's Best, Inc., San Clemente, CA) and ImmunAssure (Schiff Nutrition Group, Inc., Salt Lake City, UT) give no indication that Immunolin® is blood-derived. Interestingly, as the labeling regulations stand these manufacturers are not violating the law, as the labeling laws require them only to list the names of ingredients. Declaring the source of the ingredients is voluntary [140].

Certainly, the lax labeling regulations regarding the use of blood proteins as food ingredients, particularly in dietary supplements, fails to adequately protect individuals seeking to avoid consuming these products for religious and ethical reasons. Better labeling is vital to ensure that the interests of such people are protected and to boost consumer confidence in such products, thus promoting efforts to ensure the full-scale utilization of blood proteins.

4.3 Health reasons

The belief that blood provides a haven for pathogens and toxic metabolites is another reason why some people avoid consuming blood. This concern, though genuine, is not exclusive to blood products; in general, foods of animal origin are easily contaminated with spoilage microorganisms and possibly pathogens through improper processing and handling [141]. Blood taken from a healthy animal is essentially sterile, so any contamination must be due to the bleeding technique and the drainage system employed during collection [142]. Both manufacturers and processors have instituted measures to guarantee the safety of these blood proteins. In the US, federal regulation 9 CFR 310.20 monitors blood that enters the food chain, ensuring that it originates from official establishments whose livestock and carcasses have passed inspection. The use of closed-draining systems in the collection of blood ensures the blood is siphoned directly from the animal into collecting tanks without

exposure to the atmosphere. As a further safety measure, the collected blood is associated with the individual animals or batches of animals from which the blood was sourced until inspected to prevent uninspected blood from entering the food chain [143]. Collected and inspected blood is transported in refrigerated and dedicated isothermal stainless steel trucks to the processing plant to prevent any possible re-contamination. On arrival at the manufacturing plant, quality assurance (QA) and quality control (QC) procedures are performed on the received blood prior to spray-drying at high temperatures to inactivate any pathogens that may be present. Using Fibrimex® as an example, it is the opinion of the European Commission Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food that its use as a food additive is not of concern from the safety point of view [144].

There are situations where liquid blood (or plasma) is utilized as an ingredient in foods, although such usage is only allowed in formulations that will undergo heat-treatment. However, considering the highly perishable nature of blood, where blood is used in the liquid form extra precautions through a mandatory HACCP system may be necessary. Ramos-Clamont and others noted that the use of a HACCP system in a pilot plant ensured that isolated blood fractions had excellent microbiological quality [145]. Thus, there is still scope for more improvements to be implemented to guarantee the quality of these blood ingredients. For example, it has been argued that the use of blood proteins as binders in meat products has a relatively high risk of bacterial contamination because pathogenic and spoilage microorganisms on the surfaces of the meat and trim pieces become internalized. This is compounded by the fact that these binders work on the cold-set method. Although such health concerns arise because of the process and not the ingredients per se, they nonetheless require attention. Incorporation of bacteria growth inhibitors such as nisin (a broad-spectrum bacteriocin) in the formulation of restructured meats, which has been found to be effective in reducing undesirable bacteria in such products [146], may be one way to address this problem.

5. Conclusions

As this review has shown, blood proteins provide an economic and readily available alternative source of proteins and irons for use in foods and dietary supplements to address a wide range of functional and nutritional needs. Its additional benefits as a source of bioactive peptides with anti-hypertensive, anti-bacterial, analgesic, and antinociception properties have the potential to provide safer and cheaper alternatives to conventional drugs, which tend to be expensive and have unwelcome side effects. Efforts to ensure large-scale utilization of blood proteins as food additives should be encouraged because of the economic, nutritional, health and environmental benefits conveyed. This is particularly true in developing countries, where traditional sources of proteins and irons are either expensive or are unavailable. However, even as the industry strives to achieve greater utilization of blood as a food additive, consumer concerns related to religious and ethical issues must be addressed through the concerted effort of regulators, producers and researchers to develop accurate and concise labeling based on adequate labeling regulations and the development of effective blood detection methods to enforce regulations. On the issue of the safety of these products, making HACCP a mandatory requirement for plants that produce blood proteins, providing third world countries with the necessary expertise and equipment for

their production, and educating the public about the safety of these blood proteins, will encourage consumer confidence in these products and, consequently, their greater utilization.

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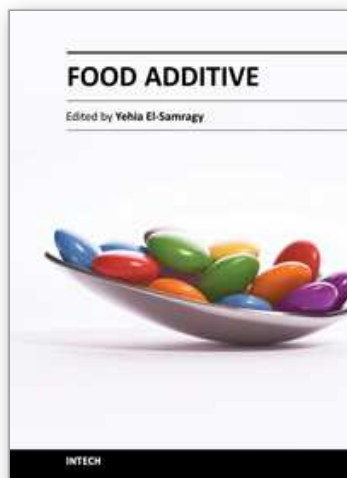
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Food Additive

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A food additive is defined as a substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value. Food additives are natural or manufactured substances, which are added to food to restore colors lost during processing. They provide sweetness, prevent deterioration during storage and guard against food poisoning (preservatives). This book provides a review of traditional and non-traditional food preservation approaches and ingredients used as food additives. It also provides detailed knowledge for the evaluation of the agro-industrial wastes based on their great potential for the production of industrially relevant food additives. Furthermore the assessment of potential reproductive and developmental toxicity perspectives of some newly synthesized food additives on market has been covered. Finally, the identification of the areas relevant for future research has been pointed out indicating that there is more and more information needed to explore the possibility of the implementation of some other materials to be used as food additives.

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